

Breadmaking properties of wheat flour supplemented with thermally processed hypoallergenic lupine flour

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Abstract

In recent years there has been increased interest in using lupine for human nutrition due to its nutritional properties and health benefits. Moreover, lupine is used as an ingredient in breadmaking because of its functional and technological properties. However, a higher number of allergic reactions to this legume have recently been reported as a consequence of a more widespread consumption of lupine-based foods. In a previous study, several thermal treatments were applied to lupine seeds and flours resulting in reduced allergenicity. In order to study how this thermal processing (autoclaving and boiling) affects the breadmaking properties, raw and thermally processed lupine flours were used to replace 10% of wheat flour. The effect of supplementing wheat flour with lupine flour on physical dough properties, bread structure and sensory characteristics were analysed. The results indicated that thermally-treated lupine flours, had similar breadmaking and sensorial properties as untreated lupine flour. These thermal treatments could increase the potential use of lupine flour as a food ingredient while reducing the risk to provoke allergic reactions.

Additional key words: allergy, *Lupinus albus*, thermal treatment.

Resumen

Propiedades de panificación de harina de trigo complementada con harina hipoalergénica de lupino obtenida por procesamiento térmico

En los últimos años se ha incrementado el interés en la utilización del lupino para la nutrición humana debido a sus propiedades nutricionales y efectos saludables. Además, el lupino se utiliza como un ingrediente en panificación por sus propiedades funcionales y tecnológicas. Sin embargo, recientemente se ha observado un aumento en las reacciones alérgicas a esta leguminosa como consecuencia de un mayor consumo de alimentos que contienen lupino. Nuestros trabajos previos indican que la aplicación de diferentes tratamientos térmicos a semillas de lupino da lugar a una disminución comprobada de su inmunoreactividad frente a IgE. Para estudiar el efecto de algunos de estos procesos térmicos (autoclavado y cocción) sobre las propiedades de panificación, se reemplazó un 10% de harina de trigo por harina de lupino cruda o procesada. Se analizó el efecto de esta adición de harina de lupino en las propiedades físicas de la masa, estructura del pan y características sensoriales. Los resultados indicaron que las harinas de lupino térmicamente tratadas, las cuales eran hipoalergénicas, mantenían sus propiedades de panificación en relación con el lupino crudo. Estos tratamientos térmicos, al reducir el riesgo de provocar reacciones alérgicas, podrían hacer posible el incremento del uso de la harina de lupino como ingrediente de los alimentos.

Palabras clave adicionales: alergia, lupino, tratamientos térmicos.

Introduction

There is growing interest in industrial exploitation of new protein sources such as plant proteins to broaden the range and variety of foods. Sweet lupine seeds (e.g. *Lupinus albus* L.) seem to be particularly promising

as a source of innovative ingredients having, on average, a protein content similar to soybean (34-43% of dry matter) and an adequate composition of essential amino acids (Yáñez *et al.*, 1983). Foods based on sweet lupine protein are gaining attention from industry and consumers because of their possible role in the prevention of cardiovascular disease as well as in reduction of blood glucose and cholesterol levels (Magni *et al.*, 2004; Sirtori *et al.*, 2004; Hall *et al.*, 2005; Duranti,

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2006). Furthermore, lupine exhibits useful techno-functional properties allowing its use as an ingredient in the production of several palatable food products, such as biscuits, pasta and bread (Drakos *et al.*, 2007) while producing satiety at the same time (Lee *et al.*, 2006). According to literature, about 10% of lupine replacement is the most convenient amount to improve breadmaking properties (Lucisano and Pompei, 1981; Doxastakis *et al.*, 2002) and the allowed upper limit by the European food authorities.

However, in the past years, an increasing number of IgE-mediated allergic reactions to lupine have been reported (Hefle *et al.*, 1994; Matheu *et al.*, 1999), especially in patients with allergy to peanut (Moneret-Vautrin *et al.*, 1999; Faeste *et al.*, 2004) and other legume species (Magni *et al.*, 2005). With the increased inclusion of lupine products in human foods, particularly in Europe, the allergenic potential of lupine seeds has become clear (Moneret-Vautrin *et al.*, 2004; Gayraud *et al.*, 2009). In fact, since November 2007 it is mandatory to label lupine and products thereof according to Directive 2007/68/EC of the European Parliament (Commission Directive, 2007). Recently, conglutin β has been identified as a major allergen in *L. albus* (Guillamón *et al.*, 2007) as well as in *L. angustifolius* (Goggin *et al.*, 2008).

Processing methods can be useful to obtain lupine products that minimize the allergenic potential. Our previous studies have demonstrated that boiling lupine seeds in an autoclave at 2.6 bar for 20 min drastically reduces or, following longer treatments, even abolishes IgE binding (Álvarez-Álvarez *et al.*, 2005). A more recent work concluded that the combination of heat and pressure is required to eliminate lupine allergenicity with treatments like instantaneous controlled pressure drop (DIC®) (Guillamón *et al.*, 2008).

The aim of this research was to study the effects of incorporating lupine flours produced from differently thermally-treated lupine seeds (cooking and autoclaving) into wheat flours on the physical dough properties and organoleptic quality of lupine-wheat breads.

Materials and methods

Materials

Sweet lupine seeds (*L. albus* var. Multolupa) were obtained from the Servicio de Investigación y Desarrollo Agrario (SIA, Badajoz, Spain). Wheat flour (*Triticum*

aestivum L.) supplied from Centro Tecnológico de Cereales (CETECE, Palencia, Spain), was used for the dough formulations and baking experiments.

Whole lupine seeds were subjected to two different treatments, autoclaving and boiling according to Álvarez-Álvarez *et al.* (2005) in order to obtain hypoallergenic flour. Using a tabletop autoclave (CertClav Multicontrol IPX4, Traun, Austria), the seeds were mixed with water (1:5 w/v) and treated at 121°C (1.18 atm) for 5 or 20 min and at 138°C (2.56 atm) for 20 min. For boiling, the seeds (1:10, w/v) were cooked in distilled water at 100°C for 30 or 60 min.

Raw and thermally processed lupine seeds were manual dehulled, frozen and then freeze-dried. The freeze-dried samples were crushed (Thermomix, Vorwerk) and milled (Mill-3303, Perten) and passed through an 800 μ m mesh sieve. The fine flour was kept in a refrigerator until analysis.

Flour blends

The six blends used in this study (Table 1) were prepared by replacing 10% of wheat flour with unprocessed lupine flour (B 1) or lupine flours thermally treated (B 2–B 6). The blends were homogenized with a mixer robot (model R6VV, Robot-Coupe) for 3 min. Wheat flour (100%) was used as control (B 0).

Chemical analysis

The nitrogen and moisture contents of wheat, raw lupine and the different wheat-lupine flour blends were determined according to standard procedures based on the AOAC methods (2002). The total protein content was calculated as $N \times 5.45$ (Mossé, 1990). All analyses were performed in duplicate.

Table 1. Formulations of different lupine-wheat blends

Blend	Wheat (g/100 g)	Lupine (g/100 g)
B 0 (control)	100	—
B 1	90	10, raw
B 2	90	10, autoclaved 121°C, 5 min
B 3	90	10, autoclaved 121°C, 20 min
B 4	90	10, autoclaved 138°C, 20 min
B 5	90	10, boiled 30 min
B 6	90	10, boiled 60 min

Table 2. Bread composition

Ingredients	g/100 g	Weight (g)
Flour	100	2,000
Water	57	1,140
Start dough	20	400
Yeast	2	40
Salt	2	40
Improver*	0.5	10

* Wheat flour, mono- and diglycerides fatty acid, monocalcium phosphate, ascorbic acid and fungal amylases.

Breadmaking procedure

Experimental breadmaking was done at the Centro Tecnológico de Cereales (CETECE) according to the procedure and recipe (Table 2) that have been developed there (Rubio *et al.*, 2008). The flours used were the different wheat-lupine blends shown in Table 1. A standard wheat bread was prepared as control (P 0) and all the breads were manufactured at similar conditions. All the ingredients were mixed with exception of the yeast that was incorporated 5 min before the end of the mixing time (13 min). Afterwards, the temperature and pH (pH-metre Crison GLP 21-22 with a pH 52-01 electrode) of the dough were measured.

The resulting dough was allowed to rest for 10 min in a cabinet at 24°C. Afterwards, it was divided into 8 pieces that were put into baking tins (21 × 8 × 8 cm) and fermented (fermentation chamber Iverpan, Industrias Alba SA) at 30°C and 80% relative humidity for 80 min. The loaves were baked at 215 ± 5°C in an electric deck oven (Tayso S.A.) for 25 min and then cooled at room temperature for 90 min. Eight loaves per flour mixture were produced. Bread characteristics and baking qualities were evaluated after cooling by measuring loaf weight, height, volume and density. Loaf volume was measured by seed displacement (AACC, 1983); density was calculated by dividing the loaf weight by its corresponding loaf volume.

Sensory evaluation

A descriptive qualitative sensory evaluation was performed by a trained expert panel using the norm UNE 87-017-092 (UNE, 1992). The attributes used in the analysis were determined by panel consensus: appearance (crust spot, crumb colour and alveolate);

bread smell (intense, roasted or torrefied, green vegetable, leguminous and cereal); bread taste (lupine, roasted or torrefied, bitter and sweet) and bread texture (gritty, chewy, soft and dense). These attributes were evaluated in six loaves of each type of bread (control and wheat-lupine blends), freshly baked and after two days.

Statistical analysis

Analysis of variance (ANOVA) was conducted for the chemical analysis data. The mean values were compared by Duncan's multiple range test.

Results

Chemical composition

The moisture and protein content was determined in raw lupine flour, wheat flour, and wheat-lupine blends. Raw lupine moisture was 8.7 g/100 g; the moisture of all blends was significantly lower than that of the wheat flour (12.6 g/100 g) ($P < 0.05$), varying between 11.7 and 12.1 g/100 g. Raw lupine protein content was 38.3 g/100 g; the protein content of the mixtures, containing 10% of lupine flour, was significantly higher (13.6-13.9 g/100 g) ($P < 0.05$) than that in the control flour (wheat) (11.3 g/100 g).

Physical properties of dough

During the kneading process it was important to control the temperature, pH level and volume of the dough in order to achieve adequate fermentation. The data obtained showed that there are no noticeable differences in the temperature (26-27°C) and the pH level (5.7-5.9). The wheat dough volume (B 0) after 13 min of kneading was 140 mL; the volumes of those supplemented with 10% of raw or heat treated lupine was similar to the control ranging between 135-150 mL.

The dough control (B 0) was easily formed with good absorption of water, resulting in smoother dough with moderate flexibility and tenacity. When wheat flour was supplemented with 10% raw lupine flour (B 1), the dough was sticky and more flexible but less smooth in texture and somewhat irregular. The dough with lupine autoclaved at 121°C for 5 min (B 2) showed

great plasticity and it was smooth and regular without signs of weakness or stickiness. When time and temperature of the autoclaved treatment were increased (B 3-B 4), the resulting dough became drier and darker. However, when cooked lupine flour (B 5-B 6) was used, the dough was more flexible and slightly sticky.

Baking properties

Figure 1 show photographs of loaves and central slices of the control bread (P 0) and the different breads made with wheat-lupine blends (P 1-P 6).

The volume, weight, density values of the loaves and the dimensions of the central slices were determined after 90 min of cooling. Eight loaves were produced from each type of dough, of which two from each batch were excluded because of their irregular appearances. The wheat flour bread (P 0) showed the highest volume (1,114 mL), lowest density (0.24 g mL^{-1}) and best performance. All breads made with a mixture of wheat-lupine had lesser volumes (1,000-1,097 mL). The maximum loss of volume was measured in the loaves produced with lupine autoclaved at 138°C for 20 min (P 4), resulting in the highest density (0.26 g mL^{-1}). The weight value of P 0 (262.5 g) was very similar to those baked with lupine flours (263.0 g).

Sensory evaluation

The main factors, defined by consensus, to determine good acceptability of bread supplemented with thermally treated lupine flour were smell and taste like roasted lupine, juicy texture and yellow colour of the crumb.

Table 3 shows the results of the descriptive sensory evaluation. The best freshly baked lupine-containing loaves were obtained after autoclaving at 121°C for 5 min as well as autoclaving at 138°C for 20 min (P 2 and P 4, respectively). P 2 was recognized for its low odor and cake taste and P 4 for had a juicy texture and more intense roasted lupine flavor. Meanwhile, the least accepted doughs were made with raw lupine (P 1) and lupine flour cooked for 60 min (P 6) resulting in poor texture and taste, respectively. In the sensory evaluation after two days of storage, the best evaluation was achieved by bread P 4 (autoclaved at 138°C for 20 min) because of its texture and flavor, and by P 5 (lupine cooked for 30 min) because of its moisture and juiciness. Bread P 6 received again the worst marks.

Discussion

Lupine flour offers a wide range of possible food ingredients as well as tasty and health-promoting foods (Magni *et al.*, 2004; Sirtori *et al.*, 2004; Hall *et al.*, 2005; Duranti, 2006). The supplementation of wheat flour with up to 10% lupine flour can result in improved nutritional and techno-functional quality of breads. However, with this inclusion lupine has been recognized as potentially allergenic, and the risk for sensitive consumers should therefore be minimized.

We have previously demonstrated that thermal treatments such as boiling and autoclaving resulted in a considerable reduction of IgE immunoreactivity of lupine flours. More precisely, autoclaving at 138°C almost abolished the IgE reactivity. It was the aim of the present study to elucidate if these hypoallergenic lupine flours (obtained by cooking or autoclaving) can be used to produce palatable mixed wheat-lupine breads of high quality.

Lupine has a high protein content (*L. albus* var Multolupa 38.3 g/100 g), which makes it a valuable supplement for wheat flour and the production of high-protein bread. However, sweet lupine flour is deficient in sulfur-containing amino acids, methionine and cystine, which are present in significant quantities in wheat flour. Therefore, a mixture of wheat and sweet lupine flour should have a higher nutritional value than the individual ingredients. The substitution of 10% of wheat flour with raw lupine flour or thermally treated lupine resulted in a significant increase in the total protein content of the flour mixture by more than 2.5%. Similar results were reported by Lucisano and Pompei (1981) and Ballester *et al.* (1984). It has also been demonstrated that the substitution of 10% wheat flour with lupine increases the lysine content in comparison to control bread (El-Dash and Campos, 1980; Mubarak, 2001).

The baking process adopted was effective. All dough mixtures were successfully baked; however, the dough containing lupine flour autoclaved at 121°C for 20 min (P3) showed rather high dough retraction.

The quality of food is determined by physical and organoleptic characteristics. In this study, the volume, weight, density and size of the loaves were measured and descriptive qualitative analysis was used to evaluate the organoleptic characteristics.

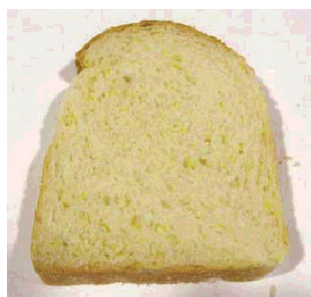
The slight loss of volume observed for blends of wheat and lupine has also been seen in previously published studies and was attributed to a decrease

in the content or quality of gluten (Campos and El-Dash, 1978; Ballester *et al.*, 1984; Dervas *et al.*, 1999; Mubarak, 2001; Doxastakis *et al.*, 2002). In the present study, the crusts of the breads made with lupine flour

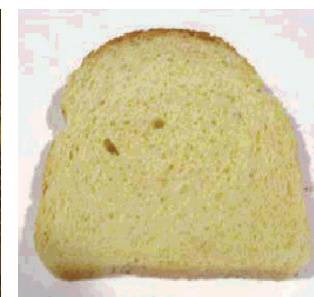
showed small pores, probably because the mesh that forms the gluten around the starch granules was weaker than in whole wheat bread. The porous crust allowed the evaporation of gases from the bread during



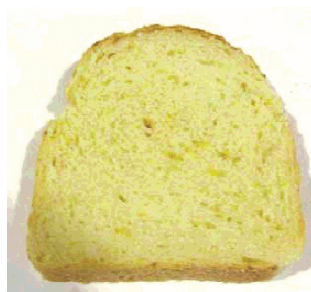
P 0



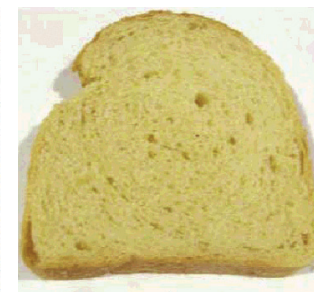
P 1



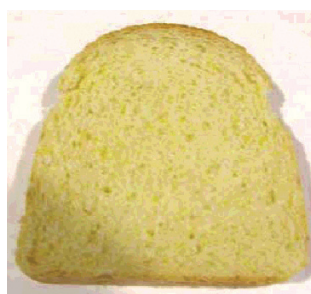
P 2



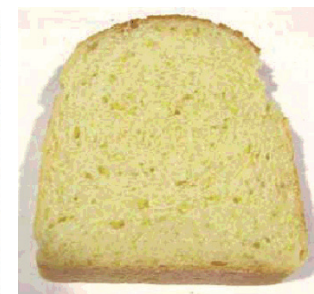
P 3



P 4



P 5



P 6

Figure 1. Whole pieces and central slices of bread made with wheat flour (P 0, control) or mixed with 10% of raw lupine flour (P 1); autoclaved 121°C, 5 min (P 2); autoclaved 121°C, 20 min (P 3); autoclaved 138°C, 20 min (P 4); boiling 30 min (P 5) and boiling 60 min (P 6).

the baking process and led to volume losses. The same results were observed by Lucisano and Pompei (1981).

According to the descriptive sensory evaluation, the bread made with wheat flour had less flavour than those

made from a mixture of wheat flour and lupine. The inclusion of lupine produces a slight lupine flavour. When autoclaved lupine flours were used it was observed that the flavour and the smell of the bread increased in parallel with autoclaving time and tempe-

Table 3. Descriptive sensory analysis of bread made with wheat flour (P 0, control) and mixed with 10% of raw lupine flour (P 1); autoclaved 121°C, 5 min (P 2); autoclaved 121°C, 20 min (P 3); autoclaved 138°C, 20 min (P 4); boiling 30 min (P 5) and boiling 60 min (P 6)

Bread	Freshly baked	Two days after baking
P 0	Smell: slightly yeasty Texture: compact, elastic and quite chewable Taste: like bread product Alveolate: uniform and closed Crumb colour: creamy white Crust colour: without blemishes	Smell: less intense than freshly baked Texture: quite fresh Taste: tasteless
P 1	Smell: slightly leguminous Texture: rough Taste: quite lupine Alveolate: open and big Crumb colour: slightly yellowish-white Crust colour: spotty	Smell: weakly lupine and green vegetable Texture: fairly fresh, a bit sticky on the palate Taste: slightly lupine
P 2	Smell: slightly leguminous Texture: juicy and soft, easy to chew Taste: like cake The taste, texture and smell are more pleasant than in P 1 Alveolate: open and big Crumb colour: yellowish-white Crust colour: spotty	Smell: almost odourless Texture: hard crust. In mouth is dry and rough Taste: tasteless
P 3	Smell: roasted lupine Texture: juicy, between P 1 and P 2 Taste: slightly lupine Alveolate: open and big Crumb colour: yellowish-white Crust colour: spotty	Smell: slightly roasted lupine Texture: hard crust. In mouth is dry Taste: weakly torrefied lupine
P 4	Smell: strong roasted lupine Texture: juicy, moist, easy to chew Taste: roasted Alveolate: open and big Crumb colour: yellow ochre Crust colour: few spots	Smell: weakly torrefied lupine Texture: fairly fresh, similar P 1 Taste: weakly torrefied lupine (more intense than P 3)
P 5	Smell: roasted lupine (similar P 3) Texture: slightly resistant to chew Taste: intense, slightly salty and weakly lupine Alveolate: open and big Crumb colour: yellowish-white Crust colour: spotty	Smell: almost odourless. Slightly damp Texture: fresh and moist in mouth Taste: low intense
P 6	Smell: slightly lupine Texture: slightly juicy Taste: weakly lupine Alveolate: open and big Crumb colour: yellowish-white Crust colour: spotty	Smell: almost odourless Texture: slightly dry and rough Taste: low intense

perature. Both, the freshly made and the two days old P 4 bread were highly valued by the expert panel.

The attributes related to the appearance of the bread (alveolate, colour of crumb and crust) remained unchanged after two days storage. According to Dervas *et al.* (1999), the alveolar structure of the crumb varies depending on the percentage of the lupine flour employed. They found that the crumb structure was acceptable in breads containing 10% lupine flour.

Lupine flour causes a yellowing due to the presence of fat-soluble pigments, primarily lutein and zeaxanthin (Feldheim, 1991; Biolley *et al.*, 2000). Although a direct relationship between the intake of these pigments and the prevention of certain eye diseases has not been proven so far, it is recommended to maintain a minimum concentration in blood (Granado *et al.*, 2003). The bread P 4 presented a relatively dark coloured crumb with a yellow ochre tone, while the other breads had yellowish-white tones. The colour of the crust and crumb darkens with increasing content of lupine flour in the dough. For some consumers, the yellow colour of the crumb would seem attractive because it resembles cakes that have been elaborated with egg (Campos and El-Dash, 1978; Dervas *et al.*, 1999; Mubarak, 2001; Doxastakis *et al.*, 2002; Pollard *et al.*, 2002). The browning of the crust is due to the Maillard reactions, which occur as a result of the increased content of protein, added when using lupine flour (El-Dash and Campos, 1980). In the present study all breads contain 10% lupine, the differences in crust colour result from the lupine processing.

Zacarias *et al.* (1985) showed that there were no significant differences in taste, aroma, texture and appearance (except colour) between breads made with wheat flour and those made with up to 12% lupine flour. These results are consistent with those obtained by Campos and El-Dash (1978), which showed that the addition of up to 10% of lupine had no negative effect on the smell or the flavour of the bread. In contrast, there was a significant loss of texture and flavour of the bread when using more than 6% of lupine flour in the study by Mubarak (2001).

In our study, we found that wheat-lupine flour blends containing thermally processed lupine resulted in very good quality loaves, with interesting sensory characteristics and good behaviour during the baking process. It is worth to notice that the evaluation panel preferred loaves made from autoclaved hypoallergenic lupine flour (P 4) (Álvarez-Álvarez *et al.*, 2005) to those made

from raw lupine or wheat due to their texture and taste. Autoclaving lupine flour at 120°C for 20 min leads to a considerable reduction in allergenicity and the prolonged treatment for 30 min at 138°C even abolished the IgE binding of major lupine allergens.

Finally, we can conclude that the use of sweet lupine flour from thermally-treated seeds in bread is feasible, and that the functional tolerance is good. Boiled and autoclaved lupine flours maintain the breadmaking properties of raw lupine flour, allowing their use in baking.

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